factor binding sites and we are now testing the ability of this element to drive reporter gene expression consistent with the expression of *Meis*2.

doi:10.1016/j.ydbio.2009.05.162

Program/Abstract # 141
The regulation of mouse Hoxb9
Tara B. Alexander, Youngwook Ahn, Robb Krumlauf
Stowers Institute of Medical Research, Kansas City, MO, USA

Hox proteins play a fundamental role in assigning anterior–posterior positional identity to segments, such as the rhombomeres and somites, during vertebrate development. Since segmental identity is affected by even small changes in spatial or temporal aspects of *Hox* gene expression, *Hox* expression must be precisely regulated and coordinated with the processes of segmentation. In this study, we focus on the regulation of *Hoxb9* in the mouse with the ultimate aim of identifying the trans-acting factors responsible for different phases of its expression. A large plasmid reporter recapitulates early *Hoxb9* expression in the neural tube and somites, but neural expression is not maintained at later stages. We have identified three independent enhancers within the larger plasmid capable of driving some aspects of *Hoxb9* expression. One of these enhancers lies within the first coding exon of *Hoxb9* and is highly conserved between zebrafish and mouse. This enhancer contains consensus binding sites for YY1, Cdx2, and TCF/Lef. These are known to play a role in the regulation of some *Hox* genes. Mutation or deletion of these sites from this enhancer implicates their involvement in the regulation of *Hoxb9*. Moreover, removal of this region from a large reporter plasmid leads to expanded mesodermal expression at early stages and expanded neural expression at later stages suggesting that this region may play a repressive role in *Hoxb9* expression. Further characterization of these enhancers should lead to additional insight into the mechanisms regulating *Hoxb9* expression during early vertebrate development.

doi:10.1016/j.ydbio.2009.05.163

Program/Abstract # 142
Counting Hox transcript numbers within single cells in fixed *Drosophila* embryos: Evidence for a stochastic mode of gene expression
Adam Pare[a], Derek Lemons[a], Dave Kosman[a], William Beaver[b], William McGinnis[a]
[a]*Department of Cell and Developmental Biology, UC San Diego, USA*
[b]*Department of Computer Science, UC San Diego, USA*

Determining mRNA concentrations within a population of cells has been a difficult problem for molecular biologists. Traditionally the problem has been addressed using Northern Blot analyses, RT-PCR, and most recently microarrays. While relatively simple to carry out these methods all have their shortcomings. Most importantly they are limited by poor temporal and spatial resolution, especially when examining complex tissues, because they necessitate the destruction of the sample. We demonstrate that FISH (fluorescent *in situ* hybridization) is capable of detecting and resolving, at extremely high efficiency, single mRNA molecules in fixed *Drosophila* embryos. We also briefly report a method for the segmentation and automated quantification of mRNA signals. The method is used to characterize and quantify the expression pattern of the Hox gene *Scr* at several stages during development. Importantly, we provide evidence for a stochastic mode of transcription, and show that levels of nascent transcription in the nucleus do not correlate well with cellular mRNA levels.

doi:10.1016/j.ydbio.2009.05.164

Program/Abstract # 143
Quantitative RT-PCR analysis of Dll-B knockdown in the ascidian *Ciona intestinalis*
Matthew D. Blanchette, Steven Q. Irvine
Department of Biological Sciences, Univ. of Rhode Island, Kingston, RI, USA

The Dll-B homeobox transcription factor in the simple chordate *Ciona intestinalis* is expressed in the entire animal hemisphere, fated to produce ectoderm, in the blastula and gastrula stages. We used transgenic siRNA and dominant negative strategies to knock down wild type CiDll-B expression. We are investigating the effects of these knockdown constructs through quantitative RT-PCR analysis of reported CiDll-B regulatory targets.

doi:10.1016/j.ydbio.2009.05.165

Program/Abstract # 144
Identification of cis-regulatory elements controlling Six6 expression
Kelley L. Crouse, Meaghan McCarthy, Andrea S. Viczian, Matthew Theisen, Michael E. Zuber
Department of Ophthalmology, SUNY Upstate Medical Univ., Syracuse, NY, USA

The homeobox-containing transcription factor, Six6, regulates eye size by stimulating retinoblast proliferation. In order to identify cis-regulatory elements controlling its expression we first identified conserved sequences in the flanking regions of the *Six6* gene. Three evolutionarily conserved regions (ECRs) were identified. ECR1 and 2 are located in the 5′ flanking region within 1.5 kb of exon 1, while ECR3 is approximately 2 kb 3′ of Six6 exon 2. We compared endogenous Six6 expression to that of eGFP in transgenic *Xenopus laevis* generated using constructs containing all three conserved regions (ECR1/2/3) and regions 1 and 2 (ECR1/2). GFP expression in ECR1/2/3 transgenics mimicked that of Six6 as it was first detected in the eye field at stage 15. By stage 24/25, eGFP was uniformly expressed throughout the developing eyes. By comparison eGFP mRNA expression was first detected in the developing eyes of ECR1/2 transgenics at st. 33/34. At stage 33/34 the expression patterns in ECR1/2/3 and ECR1/2 transgenics were similar. At later developmental stages eGFP expression remained strong in the eyes of both sets of transgenics and could be observed in the axons of retinal ganglion cells leading to their tectal targets. These results suggest ECR3 is required for early (neural plate stage) expression, while ECR1 and 2 are sufficient for the late retinal expression of Six6. Further analysis is needed to better define the role of ECR1–3 in Six6 expression. Bioinformatics and mutational analysis of these cis-regulatory regions will help identify transactivating factors controlling Six6 expression and ultimately eye size.

doi:10.1016/j.ydbio.2009.05.166

Program/Abstract # 145
The role and regulation of FoxN2/3 in the skeletogenic cells during sea urchin development
Ho Kyung Rho, David R. McClay
Department of Biol., Duke Univ., Durham, NC, USA

The primary mesenchyme cells (PMCs) form the skeleton in sea urchin larvae and provide a good model system for studying specification, cell signaling, and morphogenesis. A PMC gene regulatory network (GRN) has been built experimentally and shows the relationship between transcription factors (TFs) that direct PMC specification and skeletogenesis. Although advanced, some connections of the PMC GRN require further study. FoxN2/3, a Forkhead TF, is known to be expressed in the PMCs, but its function and regulation is not well known. Here we show FoxN2/3 plays an important role in PMC specification and ingression. Knockdown of FoxN2/3 using an antisense morpholino initially inhibits PMC ingression. Although these embryos eventually gastrulate, the resulting larval skeletons become truncated and disorganized. FoxN2/3 regulates several PMC specific genes involved in the biomineralization including Sm30 and Sm50. FoxN2/3 expression begins at the early blastula stage in the vegetal plate (the territory of the PMCs) but is no longer detectable after ingression. FoxN2/3 expression shifts in the vegetal plate to the non-skeletogenic mesoderm and later to the endoderm. This moving torus pattern of gene expression is similar to that of Blimp1b whose regulatory mechanism is well known. However, the knockdown either of Blimp1b or FoxN2/3 suggests that they have independent regulatory subcircuits. Perturbation experiments show FoxN2/3 is regulated by Pmar1, one of the most upstream TFs of the PMC GRN, and Tbr, a T-box TF. In summary, our findings suggest that FoxN2/3 plays a crucial role in PMC specification and ingression and is regulated by early acting PMC specification genes.

doi:10.1016/j.ydbio.2009.05.167

Program/Abstract # 146
Sea urchin embryonic skeletogenesis is regulated by microRNA
Daniel C. McIntyre, David R. McClay
Biology Department Duke University, Durham NC, USA

The Gene Regulatory Network (GRN) driving the first 30 h of embryonic development in the sea urchin is at an advanced state of understanding. Most of the transcription factors and signaling mechanisms active in the embryo are included in the current endomesoderm and ectoderm GRN models. As yet, however, there has been little work done on the role of post-transcriptional regulation in determining GRN behavior. Of particular interest are the roles miRNA may play in modulating the function of the GRN. miRNAs are short (~21–23nt) noncoding RNAs that negatively regulate the translation of target mRNAs by binding to their 3′ UTR. Recent studies demonstrated that miRNA function in later developmental events, but there are few studies examining miRNA function in early embryonic processes. All the genes necessary for miRNA synthesis and function are expressed in the sea urchin embryo, and the sea urchin genome contains several hundred predicted miRNA sequences. To begin to understand the role of miRNA in sea urchin embryogenesis, we characterized embryos in which Dicer (a protein required for miRNA synthesis) expression was knocked down using morpholino antisense oligonucleotides. In these embryos, development is delayed, and formation of the embryonic skeleton is completely blocked, demonstrating a requirement for post-transcriptional regulation in the skeletogenic lineage. We are currently characterizing the molecular basis of this phenotype, using the known transcription factors and signaling mechanisms in the skeletogenic GRN as a guide in order to determine exactly how miRNA regulation affects development in the early embryo.

doi:10.1016/j.ydbio.2009.05.168

Program/Abstract # 147
Developmental timing genes identified through miRNA suppressor screens in *C. elegans*
Tamar Resnick, Sarah Malmquist, Ann Rougvie
Department of Genetics, Cell Biology, and Development, University of Minnesota, Minneapolis, MN, USA

In the formation of an organism from a single cell, developmental events must be temporally regulated to ensure proper patterning of the adult. In *Caenorhabditis elegans*, timing is regulated by the heterochronic genes, and disruption of these genes leads to skipping or reiteration of certain developmental events. Among these genes are the first-described miRNAs, lin-4 and let-7. Loss of function for either of these genes causes a “retarded” phenotype in which developmental events are reiterated in subsequent stages, delaying differentiation of adult tissues. Several miRNAs, including miR-48, miR-241, and miR-84, share identity with the 5′ end of let-7 miRNA, and may target an overlapping set of mRNAs. Disruption of these three let-7 sisters together results in a retarded phenotype. Gain-of-function alleles of mir-48 were recovered as suppressors of lin-4 retarded phenotypes, and cause a precocious phenotype. Overexpression of mir-48 from multicopy arrays leads to enhanced precocious defects, including aberrations in vulva precursor cell divisions, resulting in disruption of egg-laying. To identify additional players in the pathway, we screened for suppressors of mir-48 overexpression that restored egg-laying. These screens are expected to identify new heterochronic mutants, miR-48 target genes, and genes involved in miRNA expression and function. We isolated 36 suppressed lines from 48,000 haploid genomes screened. Preliminary analysis indicates some of the suppressor alleles on their own result in retarded phenotypes, validating this approach for identification of new regulators of developmental timing.

doi:10.1016/j.ydbio.2009.05.169

Program/Abstract # 148
The effects of resveratrol on lifespan extension and gene expression in *Drosophila melanogaster*
Neha Sirohi[a], Alexis Nagengast[b]
[a]*Department of Biology, Widener University, Chester, PA, USA*
[b]*Department of Biochemistry, Widener University, Chester, PA, USA*

Caloric restriction extends lifespan in a variety of organisms including mice, worms, yeast and flies. The polyphenol resveratrol can be found in the skin of grapes and several studies have shown that dietary exposure mimics caloric restriction. Resveratrol may extend life span by activating the NAD+ dependent deacetylase sirtuins although its role on gene expression remains unclear. To replicate published studies and further understand the effect of resveratrol on gene expression during lifespan extension, we are exposing *Drosophila melanogaster* to 0 μM, 100 μM and 350 μM concentrations of resveratrol in food. Survival curves are being generated for isogenized adult males and microarray analysis will be conducted to identify genes that are differentially expressed under the various concentrations of resveratrol.

doi:10.1016/j.ydbio.2009.05.170

Program/Abstract # 149
Downstream targets of Atoh1 (Math1)
Helen C. Lai, Jane E. Johnson
Department of Neuroscience, UT Southwestern Medical Center, Dallas, TX, USA